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GENETIC BASIS OF ENDOCRINE DISEASE

Mutations in G Proteins and G Protein-Coupled Receptors in Endocrine Disease

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G Proteins couple receptors for diverse extracellular signals to effectors such as enzymes and ion channels. The majority of polypeptide hormones, all monoamine neurotransmitters, PGs, and even extracellular Ca^{2+} signal their target cells through G protein-coupled receptors (GPCR). In the past few years, mutations in G proteins and GPCR have been identified as the causes of several endocrine diseases. Understanding the molecular basis of these diseases provides valuable insights into G protein and GPCR structure and function, and may be important for diagnosis and treatment. In this paper, I briefly describe G protein-coupled signal transduction, provide a general framework for understanding how G protein and GPCR mutations cause endocrine disease, review specific endocrine disorders caused by G protein and GPCR mutations, and speculate on implications of this work for the etiology, diagnosis, and treatment of endocrine disorders.

G Protein-coupled signal transduction: a brief overview of structure and function

For detailed coverage of the enormous body of data on the structure and function of G proteins and GPCR, the interested reader is referred to several recent monographs (1, 2) and reviews (3, 4). Due to limitations of space, here I will give only a brief overview, emphasizing some features relevant to the pathophysiology of endocrine disorders caused by defective G protein-coupled signal transduction.

GPCR comprise a superfamily sharing a common structural and functional motif (1, 3, 5), a single polypeptide with seven membrane-spanning domains. All GPCR act by promoting the release of tightly bound GDP from G protein α-subunits, thereby enabling GTP to bind and activate the G protein (Fig. 1). Within the GPCR superfamily, differences in sequence and structure presumably contribute to differences in ligand recognition and G protein coupling. For small ligands such as catecholamines, the binding site is within the membrane bilayer in a pocket formed by several of the membrane-spanning domains. For larger polypeptide hormones, the extracellular amino-terminus and one or more extracellular loops may be involved in ligand binding. Different classes of GPCR couple exclusively or preferentially to specific G proteins. G protein coupling involves the intracellular loops and intracellular carboxy-terminus of the receptor.

A plausible model of GPCR function suggests that GPCR are dynamic proteins, moving spontaneously between conformations favoring and not favoring G protein coupling. According to this model, binding of a hormone agonist would stabilize the conformation favoring G protein coupling, thus activating the receptor. Certain antagonists, termed inverse agonists, not only compete with agonists for receptor binding, but may actually stabilize the inactive conformation of the receptor, thus blocking G protein activation (6).

G proteins consist of three distinct polypeptide gene products (2, 4). The α-subunit binds guanine nucleotides with high affinity and specificity. The β- and γ-polypeptides are tightly, but noncovalently, associated in a functional dimer subunit. The heterotrimer, associated with the inner surface of the plasma membrane (Fig. 1), is required for high affinity coupling to GPCR. Upon α-subunit binding of GTP and dissociation from the $\beta\gamma$-dimer, each subunit can independently modulate the activity of one or more effectors, such as adenylyl cyclase (the enzyme that generates the second messenger cAMP), other second messenger-generating enzymes, and ion channels. There are 16 mammalian α-subunit genes. They vary in range of expression and specificity of receptor-effector coupling. Some, such as $G_s\alpha$, which is responsible for stimulation of cAMP formation, are expressed ubiquitously. Based on the degree of amino acid identity, they have been divided into 4 subfamilies: G_s, G_q, G_i, and G_{12}. Members of the G_s and G_q subfamilies, in general, mediate stimulatory events such as hormone secretion, whereas members of the G_i subfamily generally mediate inhibition of processes such as hormone secretion. The physiological roles of members of the G_{12} subfamily and, indeed, the receptors and effectors they couple have not yet been clearly defined.

Mutations in G proteins and G protein-coupled receptors: general considerations

Defective G protein-coupled signal transduction could result from quantitative and/or qualitative changes in GPCR

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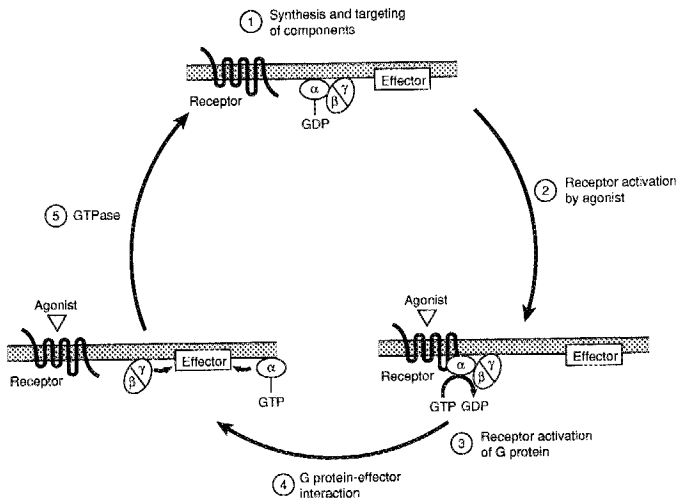

FIG. 1. The G protein GTPase cycle. Potential sites for disease-causing abnormalities are numbered. In each panel, the *stippled region* denotes the plasma membrane, with the extracellular area *above* and the intracellular area *below*. In the basal state, the G protein is a heterotrimer with GDP tightly bound to the α-subunit. The agonist-activated receptor catalyzes the release of tightly bound GDP that permits GTP to bind. The GTP-bound α-subunit dissociates from the $\beta\gamma$-dimer. *Arrows* between GTP-bound α-subunit and effector and between $\beta\gamma$-dimer and effector indicate the regulation of effector activity by the respective subunits. Under physiological conditions, effector regulation by G protein subunits is transient and is terminated by the GTPase activity of the α-subunit. The latter converts bound GTP to GDP, thus returning the α-subunit to its inactivated state with high affinity for the $\beta\gamma$-dimer, which reassociates to again form the heterotrimer.

and G proteins, including changes in the level of expression or in posttranslational modifications. A number of studies describe alterations in signal transduction in conditions such as hypertension and aging. Altered signal transduction has been ascribed to quantitative changes in G protein expression or qualitative changes in receptor-G protein coupling (7, 8). Generally, however, the observed changes in signal transduction are modest, and the molecular basis for such changes has not been clearly defined. The pathophysiological significance of such subtle differences in G protein (or GPCR) expression and function remains to be proven.

In contrast, mutations in G proteins and GPCR have been identified that unequivocally alter signal transduction with clear-cut, and sometimes devastating, pathophysiological consequences. There are three principal determinants of the phenotypic expression of such mutations: 1) the range of expression of the mutated gene; mutations in a ubiquitously expressed gene such as $G_s\alpha$, in general, will cause more generalized manifestations than those caused by mutations in a gene such as the LH receptor, which is more restricted in expression (but see the following); 2) germ-line (inherited) *vs.* somatic (postzygotic) mutations; the former potentially cause manifestations in every cell in which the gene is expressed; somatic mutation of even a ubiquitously expressed gene, in contrast, would still lead to manifestations that are localized to the cells derived from the progenitor in which the original somatic mutation occurred; and 3) the nature of the mutation; mutations can be broadly divided into those causing gain and those causing loss of function.

Loss of function mutations block normal messenger ribonucleic acid and/or protein synthesis, prevent the synthesized protein from reaching its normal subcellular location (the plasma membrane in the case of GPCR and G proteins; see no. 1 in Fig. 1), or impair function despite synthesis and normal targeting of the protein. Many GPCR mutations, including missense mutations, will cause abnormal folding of the protein with retention in the endoplasmic reticulum. Such trafficking mutations of rhodopsin, the GPCR mediating visual transduction, have been well described as a cause of retinitis pigmentosa (9), but undoubtedly also occur in G protein-coupled hormone receptors. GPCR mutations compatible with normal protein synthesis and trafficking may, nonetheless, cause loss of function by impairing agonist binding to or activation of receptor (no. 2 in Fig. 1) or by impairing receptor coupling to activation of G protein (no. 3 in Fig. 1). G protein loss of function mutations, in addition to impairing normal protein synthesis or trafficking, may block receptor coupling or activation by GTP (no. 3 in Fig. 1) or impair effector interaction (no. 4 in Fig. 1).

Gain of function mutations cause inappropriate or constitutive activation. A constitutively activated GPCR signals G protein activation, even without the hormone binding that normally activates the receptor. Likewise, a constitutively activated G protein signals its effector despite the lack of normal upstream signals from a hormone-activated GPCR. Such mutations could either accelerate the release of GDP and lead to receptor-independent G protein activation (no. 3 in Fig. 1) or block the guanosine triphosphatase (GTPase) reaction that terminates G protein activation (no. 5 in Fig. 1). Gain of function mutations are by definition dominant, and thus, heterozygotes for germ-line mutations will be clinically affected. For loss of function mutations, the situation is more complex. Pure loss of function mutations may cause no overt clinical dysfunction in heterozygotes. For many GPCR, there is sufficient signal sensitivity and amplification such that a 50% reduction in receptor number does not lead to clinically apparent disease. Certain loss of function mutations may act as dominant negatives and thereby also cause clinical disease in heterozygotes. In theory, a mutant GPCR that binds to its cognate G protein but fails to activate it or a mutant G protein that binds receptor or effector without resultant activation could act as dominant negatives, but definite examples of such mutations of either GPCR or G proteins as a cause of disease have yet to be identified.

Loss of function mutations are generally associated with inherited disorders, whereas gain of function mutations may occur in the germ line in inherited disorders or as somatic events in sporadic disorders. In the latter case, a gain of function mutation confers a proliferative advantage on the cell in which the somatic event occurs, leading to a clonal neoplasm and eventually clinically evident disease. Germ-line mutations of certain GPCR and G proteins may never be detected simply because they would be incompatible with life. This could be true of both heterozygous gain of function mutations and homozygous null mutations in which inappropriate signal activation or total lack of signaling, respectively, would be lethal. When such germ-line mutations are compatible with life, the timing of onset of clinical disease may be quite variable even though the mutation is already

present at birth. For gain of function mutations, the timing of disease onset may reflect several factors, including the degree of constitutive activation of the particular mutation, critical developmental events such as cell proliferation necessary for a response to signal activation, and the eventual failure of mechanisms attempting to compensate for inappropriate signal activation.

Mere identification of sequence variation in a GPCR or G protein gene does not constitute proof of a disease-causing mutation. Some sequence differences may simply be polymorphisms of no pathophysiological consequence (but see later discussion for possible role of polymorphisms in predisposing to disease). Identification of mutations only in affected members of a kindred and not in unaffected members or in normal control subjects suggests pathophysiological relevance. Also, certain mutations may be predicted to be functionally significant, *e.g.* those causing truncation of a GPCR, based on available information on GPCR and G protein structure and function. More rigorous proof that an identified mutation is responsible for the disease being studied requires mutagenesis of the normal gene and appropriate functional studies of the expressed gene product.

The majority of endocrine diseases can be classified into those involving either hypo- or hypersecretion of a given hormone or hormones. G protein and GPCR mutations can cause diseases of either type. Loss of function mutations of a given GPCR or of the G protein to which it is coupled will cause hormone resistance, with a clinical phenotype closely resembling that caused by deficiency of the hormone normally activating the corresponding receptor(s). Hormone resistance caused by GPCR or G protein loss of function mutations is characterized by increased circulating concentrations of the corresponding hormone agonist. Gain of function mutations will lead to a state resembling hypersecretion of the hormone normally activating the involved GPCR, but circulating concentrations of the involved hormone will actually be suppressed, reflecting autonomous hyperfunction of the target gland. In the following sections, specific diseases caused by GPCR and G protein mutations are described. Space does not permit a comprehensive listing of each individual mutation, but selected examples will be cited. For a detailed listing of mutations in G proteins and GPCR identified to date (note that new ones are continually being reported), the reader is referred to several recent reviews (1, 10, 11).

Endocrine Diseases Caused by GPCR Mutations

Loss of function mutations

Germ-line loss of function mutations in the ACTH, TSH, GHRH, FSH, LH, and V2 vasopressin receptors have been identified as causes of inherited resistance to each of the corresponding hormones. In the case of the CaR, germ-line loss of function mutations cause inherited insensitivity to extracellular Ca^{2+}. The diseases caused by loss of function mutations of the ACTH, TSH, GHRH, LH, and FSH receptors (Table 1) are transmitted as autosomal recessives, *i.e.* require homozygous or compound heterozygous mutations for clinical expression. The clinical manifestations of each of these diseases reflect the localized expression of the involved receptor.

Loss of function mutations of both ACTH receptor alleles causes adrenocortical resistance to ACTH with resultant manifestations of relative glucocorticoid deficiency, such as hypoglycemia and frequent infections (12, 13). A variety of missense, nonsense, and frame shift mutations of the ACTH receptor gene have been reported in affected members of kindreds (12, 13). The adrenocortical ACTH receptor is only one of several melanocortin receptors that bind ACTH and MSH (14). The hyperpigmentation seen in familial ACTH resistance reflects stimulation of skin MSH receptors by raised circulating ACTH. In some families, ACTH-resistant glucocorticoid deficiency is associated with alacrima and achalasia of the esophagus (triple A syndrome). Mutation of the ACTH receptor does not appear to be the cause of this syndrome or of familial isolated glucocorticoid deficiency in kindreds in which the disorder is not linked to chromosome 18p (the site of the ACTH receptor gene) (15).

A homozygous loss of function mutation in a highly conserved proline in the fourth transmembrane domain of the TSH receptor was identified as the cause of TSH-resistant hypothyroidism in the *hyt* mouse (16). Subsequently, an autosomal recessive form of congenital hypothyroidism was reported in three sisters in whom distinct missense mutations in the extracellular amino-terminus of the TSH receptor were identified (17). Each affected sister inherited a mutant paternal (asparagine 167 to isoleucine) and maternal (proline 162 to alanine) allele that together (compound heterozygosity) were sufficient to cause chemical hypothyroidism (markedly elevated TSH but normal thyroid hormones), but individually (*i.e.* in mother or father) caused only borderline TSH

TABLE 1. Endocrine diseases caused by GPCR mutations

Receptor	Disease	Mutation type
V2 vasopressin	Nephrogenic diabetes insipidus	Loss
ACTH	Familial ACTH resistance	Loss
GHRH	Familial GH deficiency	Loss
FSH	Hypergonadotropic ovarian dysgenesis	Loss
LH	Male pseudohermaphroditism	Loss
TSH	Familial hypothyroidism	Loss
CaR	Familial hypocalciuric hypercalcemia/neonatal severe primary hyperparathyroidism	Loss
LH	Familial male precocious puberty	Gain
TSH	Sporadic hyperfunctional thyroid nodules	Gain
TSH	Familial nonautoimmune hyperthyroidism	Gain
CaR	Familial hypoparathyroidism	Gain
PTH/PTHrP	Jansen metaphyseal chondrodysplasia	Gain

elevation. The functional effect of these mutations, both in the large extracellular amino-terminus characteristic of glycoprotein hormone receptors, emphasizes the importance of this domain in receptor function.

The GHRH receptor offers another example of a loss of function mutation first identified as a cause of disease in a mouse mutant and then recognized in humans. The *little* mouse shows GH-deficient dwarfism caused by a loss of function mutation in a highly conserved residue (aspartate 60 to glycine) in the extracellular amino-terminus of the GHRH receptor (18). In humans, familial isolated GH deficiency may be caused by a mutation in the GH gene itself, but in some kindreds, the disease is not linked to the GH gene locus. In such a kindred, a homozygous missense mutation (glutamate 72 to stop) in the GHRH receptor was identified in two severely GH-deficient children, both products of consanguineous marriages (19). This mutation would disrupt receptor function by severely truncating the receptor protein before the first transmembrane domain. The affected subjects failed to secrete GH in response to GHRH, but were responsive to GH replacement therapy. It was suggested that stimulators of GH release that act through mechanisms not involving the GHRH receptor, *e.g.* nonpeptidyl benzazepines, might be effective treatment in this disorder (19).

Loss of function mutations in the receptors for FSH and LH cause distinct syndromes of gonadal dysfunction, hypergonadotropic ovarian dysgenesis, and male pseudohermaphroditism, respectively. Recognition that some cases of ovarian dysgenesis show autosomal recessive inheritance and are linked to chromosome 2p (site of the FSH receptor gene) led to identification of a homozygous missense mutation (alanine 183 to valine in the extracellular amino-terminus) in the FSH receptor in affected subjects (20). Expression of the mutated gene revealed a major deficit in binding capacity and signal transduction. Clinically, females with the homozygous mutation have a normal XX karyotype, normal prepubertal development with normal internal and external genitalia, variable secondary sex characteristics, and primary amenorrhea (20). Resistance to gonadotropins at puberty (despite secondarily elevated concentrations) leads to arrest of follicular maturation, with poorly developed streak ovaries. Heterozygous females are clinically unaffected. Although homozygous mutant males have been identified in affected kindreds, the phenotypic consequences are not clear-cut and may vary from normospermia with normal fertility to azoospermia with infertility. This phenotypic variability in males is compatible with other observations suggesting that FSH action is not absolutely required for spermatogenesis. The incidence of FSH receptor mutations as a cause of hypergonadotropic hypogonadism (manifesting as primary amenorrhea in females and azoospermia in males) clearly deserves further study.

A homozygous loss of function mutation of the LH receptor (alanine 593 to proline at the junction of the sixth transmembrane domain and the third extracellular loop) was identified in two siblings, products of a consanguineous marriage, with male pseudohermaphroditism (21). Both individuals presented as XY females with Leydig cell hypoplasia caused by LH unresponsiveness. There may be milder clinical forms of this disorder, such as males with hypergonadotropic hypogonadism and micropenis. The phenotypic consequences in 46,XX females are not completely clear, but could include primary amenorrhea caused by LH resistance (21).

Nephrogenic diabetes insipidus (NDI) is characterized by failure to concentrate the urine despite adequate, indeed elevated, secretion of vasopressin. There are at least two familial forms inherited in X-linked and autosomal recessive fashion, respectively (22). The V2 vasopressin receptor, one of at least three vasopressin receptor subtypes, is expressed primarily in the kidney and mediates the antidiuretic action of the hormone. The clotting factor response to vasopressin is also a V2 response and is also defective in males with X-linked NDI. Other actions of vasopressin, such as the hypertensive response and ACTH release, are mediated by V1a and V1b receptor subtypes, respectively, and are normal in subjects with NDI. Thus, the V2 vasopressin receptor gene was already an excellent candidate gene for X-linked NDI. Localization of the V2 vasopressin receptor gene to the same chromosomal site, Xq28, as the X-linked form of NDI immediately prompted study of this receptor in subjects affected with this form of the disorder.

To date, more than 70 distinct loss of function mutations have been identified in many different kindreds (11, 22). These include nonsense, frame shift, and missense mutations involving all regions of the receptor. A single loss of function mutation causes clinically evident disease in males, not because of a dominant negative effect, but because males are hemizygous for the receptor gene. Most females heterozygous for the mutant receptor gene are clinically unaffected carriers, as random X-inactivation results, on the average, in only a 50% reduction in normal receptor number. Rarely, female heterozygotes show clinically apparent diabetes insipidus, presumably due to unfavorable X-inactivation (23). The plethora of mutations identified in X-linked NDI reflects in part the ease of transmission of the disease by carrier females, the ease of disrupting receptor function with mutations almost anywhere in the receptor, and a clinically obvious phenotype that brings affected males to medical attention. Autosomal recessive NDI phenotypically resembles the X-linked disease, but is caused by loss of function mutations in the aquaporin-2 gene (22). This gene encodes a renal tubular membrane water transporter that is the distal target of vasopressin-stimulated cAMP action.

Heterozygous loss of function mutations in the CaR gene (localized to chromosome 3q) have been identified as the cause of familial hypocalciuric hypercalcemia (FHH; also termed familial benign hypercalcemia) (24). Some families with FHH do not show linkage to chromosome 3 (25, 26) implying that mutations in genes other than the CaR may cause a similar phenotype. The CaR is comprised of an approximately 600-residue extracellular amino-terminus, thought to be the site of Ca^{2+} binding, tethered to a seven transmembrane-spanning structure typical of GPCR (27). Mutations causing FHH have been identified both in the extracellular amino-terminus as well as in the transmembrane and connecting loop portions of the receptor (28). The CaR is expressed in parathyroids, kidney, and C cells of the thyroid and brain, and is activated by Ca^{2+} in the millimolar range (28). Activation of the receptor in the parathyroid

inhibits PTH secretion. In the kidney, CaR activation by increased extracellular Ca^{2+} presumptively increases renal calcium excretion. Loss of function of a single CaR allele in FHH causes relative insensitivity to Ca^{2+} in parathyroid and kidney, with resultant increased serum Ca^{2+}, inappropriately high PTH secretion, and inappropriately low urinary calcium excretion. The impact of CaR dysfunction in other sites of receptor expression in subjects with FHH is not yet clear.

FHH is generally a benign disease, with little if any morbidity caused by the serum Ca^{2+} elevation. In contrast, loss of function of both CaR alleles causes a serious disease, termed neonatal severe primary hyperparathyroidism (NSPHT). Subjects with NSPHT may suffer lethal hypercalcemia if not treated with total parathyroidectomy. NSPHT is caused by homozygous CaR gene mutation in some infants who are products of consanguineous marriages (29, 30). Knock-out of the CaR gene in mice causes an FHH-like syndrome in heterozygotes and is lethal in the early postnatal period in homozygotes (31). Not all cases of NSPHT are due to homozygous CaR mutation; some may be compound heterozygotes, and in some, other factors may be involved. Two cases of NSPHT due to *de novo* heterozygous CaR mutations were reported, prompting the researchers to speculate about a dominant negative effect of the mutation, but the phenotype was milder in these cases with serum calcium elevation much less marked than in subjects with homozygous mutation of the CaR (32). A homozygous missense mutation (proline 40 to alanine) was identified in a Japanese subject with relatively benign (FHH-like) disease (33). The consanguineous parents showed only borderline serum Ca^{2+} elevation. Although the functional significance of the mutation was not defined by expression studies, the researchers speculated that a spectrum of mutations exists such that those causing a milder degree of loss of function may cause relatively benign disease even in the homozygous form. Given the link between CaR mutations and abnormal parathyroid function in FHH and NSPHT, it is possible that CaR dysfunction is involved in some forms of sporadic hyperparathyroidism. A study of 44 parathyroid tumors, including adenomas, carcinomas, and hyperplasia, however, failed to reveal somatic mutations in the CaR gene (34).

Gain of function mutations

After site-directed mutagenesis of adrenergic receptors showed that missense mutation of critical residues in the third intracellular loop caused constitutive activation (35), similar, naturally occurring mutations were identified in the receptors for LH (36) and TSH (37) (Table 1). Activating mutations of the LH receptor cause familial male precocious puberty, an autosomal dominant disorder in which affected males show signs of virilization often by age 4 yr or even earlier. The disease may also occur sporadically (38) as either a new germ-line mutation or a somatic event. Gonadotropins are suppressed compatible with autonomous gonadal hyperfunction. Aspartate 578 (in the sixth transmembrane domain) to glycine is a common missense mutation found in familial male precocious puberty, but a number of other missense mutations have been identified (10). An aspartate 578 to tyrosine mutation was identified in a subject with onset of precocious puberty by 1 yr of age (39). This mutant causes even greater cAMP stimulation than other constitutively activated LH receptor mutants tested in transfected cells, suggesting a correlation between degree of constitutive activation and timing of disease onset. Interestingly, there are no apparent clinical manifestations in obligate female carriers of the mutant receptor gene. Evidently, LH receptor activation of cAMP formation in testicular Leydig cells is sufficient to stimulate testosterone production and even spermatogenesis, but is ineffective in triggering puberty in females without concomitant FSH action.

Constitutive activation of the TSH receptor is caused by somatic mutations identified in hyperfunctioning thyroid adenomas (toxic or "hot" nodules) (37) and by germ-line mutations in familial nonautoimmune hyperthyroidism (40, 41). TSH is suppressed as expected with autonomous thyroid hyperfunction. In the reported pedigrees with familial hyperthyroidism, clinical onset of disease ranged from 18 months to adulthood. Aggressive ablative treatment was required to avoid relapse of this form of disease (42). In transgenic mouse models of hyperthyroidism caused by constitutive receptor activation, foci of transformed thyrocytes have been found in older animals, raising the question of whether chronic stimulation of the cAMP cascade can lead or predispose to malignant transformation (42). Identification of an alanine 623 to serine mutation of the TSH receptor in three well differentiated thyroid carcinomas suggests that this may indeed be possible (43). Whereas activating LH receptor mutations tend to cluster in the sixth transmembrane domain and adjacent intracellular loop, activating TSH receptor mutations are dispersed more widely, suggesting that the TSH receptor is intrinsically more susceptible to activation (42).

Activating GPCR mutations have also been identified as causes of inherited forms of hypo- and hypercalcemia. In the former, missense mutations of the CaR were found in an autosomal dominant form of hypoparathyroidism with mild hypocalcemia (44, 45). Expression of one such mutation, glutamate 128 to alanine, showed increased sensitivity to activation by Ca^{2+} (44). Presumptively, receptor activation at inappropriately low serum Ca^{2+} inhibits PTH secretion, causing hypoparathyroidism. Because of the expected effect of CaR activation in the kidney, one would predict inappropriately high renal calcium excretion in affected individuals.

A heterozygous activating mutation of the PTH/PTHrP receptor gene (histidine 223 to arginine in the first intracellular loop) was identified in a subject with a rare form of dwarfism, termed Jansen-type metaphyseal chondrodysplasia (46). This disease is associated with PTH-independent hypercalcemia consistent with constitutive PTH receptor activation mimicking the effect of a systemic increase in PTH. Abnormal endochondral bone formation in this disorder reflects the critical role of the PTH/PTHrP receptor in normal proliferation and differentiation of growth plate chondrocytes (47).

Endocrine Diseases Caused by G Protein Mutations

Loss of function mutations

Pseudohypoparathyroidism (PHP) was the first disorder recognized to be caused by hormone resistance rather than hormone deficiency. PHP is characterized by normal renal function, hypocalcemia, and hyperphosphatemia despite increased PTH. There are several forms of the disease. Most subjects with PHP fail to show the normal rise in urinary cAMP excretion after PTH administration (PHP type I), suggesting a defect in signal transduction proximal to second messenger generation. In the form termed PHP type Ib, hormone resistance is limited to PTH. This is consistent with the possibility of a defect in the PTH receptor, such as a loss of function mutation, comparable to those seen in other hormone resistance disorders (Table 1). This hypothesis was not supported by a study of the PTH receptor in 18 subjects with PHP Ib (47). Knock-out of the receptor gene in mice causes a lethal phenotype in homozygotes characterized by severe chondrodysplasia (47). A homozygous total loss of function mutation of the PTH receptor, then, is not likely to be a cause of PHP Ib, as this might be a lethal genotype in humans as well. It remains possible that other defects in receptor function could cause this disorder (48).

In the subtype termed PHP Ia (Table 2), affected subjects show resistance not only to PTH, but also to several other hormones, such as TSH and gonadotropins, whose actions are mediated by receptors coupled to G_s with resultant cAMP stimulation. Affected individuals also show phenotypic features, obesity, short stature, and skeletal abnormalities, collectively termed Albright's hereditary osteodystrophy (AHO). Within a kindred with AHO, certain individuals may show these phenotypic features without hormone resistance (so-called pseudo-PHP). A variety of loss of function mutations in the $G_s\alpha$ gene have been identified in kindreds with AHO (see Ref. 2 and references therein). These heterozygous mutations cause an approximately 50% reduction in functional $G_s\alpha$ protein, which is thought to impair the cAMP response to hormone stimulation. A germ-line mutation in this ubiquitously expressed gene offers a ready explanation for the pleiotropic clinical manifestations, but fails to explain a number of observations, including 1) the variability in hormone resistance (*e.g.* typically clinically obvious for PTH and TSH, but not for vasopressin or ACTH); and 2) the occurrence of the identical $G_s\alpha$ gene mutation within a kindred in subjects with PHP and pseudo-PHP. The latter lack hormone resistance altogether (note that a mutant $G_s\alpha$ gene is never found in completely unaffected individuals within such kindreds). It appears that a heterozygous loss of function mutation of the $G_s\alpha$ gene is necessary, but not sufficient, for full expression of the PHP Ia phenotype. Recent generation of a mouse $G_s\alpha$ gene knock-out model (Weinstein, L. S., personal communication) may help clarify some of these issues.

Two males have been reported with typical features of PHP Ia, but, in addition, with gonadotropin-independent precocious puberty. A germ-line alanine 366 to serine mutation of the $G_s\alpha$ gene was identified in both subjects (49). Expression of the mutant protein showed that it was rapidly degraded at 37 C (*i.e.* a loss of function mutation), but that at 32 C, the protein was constitutively activated due to receptor-independent GDP release (step 3 in Fig. 1). This suggested that the clinical features of PHP Ia are due to the $G_s\alpha$ loss of function mutation, whereas at the lower temperature prevailing in the testes, the mutant $G_s\alpha$ is activated, rather than degraded, and causes precocious puberty by stimulating Leydig cell cAMP formation (49).

TABLE 2. Endocrine diseases caused by G protein α-subunit mutations

G Protein α-subunit	Disease	Mutation type
α_s	PHP type Ia	Loss
α_s	PHP Ia with precocious puberty	Loss/gain
α_s	Acromegaly, hyperfunctional thyroid nodules, McCune-Albright syndrome	Gain
α_{i2}	Ovarian and adrenalcortical tumors	Gain

PHP, Pseudohypoparathyroidism.

Gain of function mutations

Activating heterozygous mutations of the $G_s\alpha$ gene have been identified in pituitary somatotroph tumors in subjects with acromegaly and in hyperfunctioning thyroid adenomas (50). These occur as somatic, rather than germ-line, events, explaining the focal disease manifestations. About 40% of tumors from subjects with acromegaly show $G_s\alpha$-activating mutations (51). Hyperfunctioning thyroid adenomas may be caused by either activating TSH receptor mutations (see earlier) or activating $G_s\alpha$ mutations. The former may predominate (42), but a recent study of 37 thyroid adenomas found only 3 TSH receptor mutations and 9 $G_s\alpha$ mutations (52). The $G_s\alpha$ gene-activating mutations identified to date involve either arginine 201 or glutamine 227. These residues are critically involved in GDP/GTP binding, and mutation of either inhibits G protein GTPase activity, causing constitutive activation. These residues are highly conserved in all G protein α-subunits, and naturally occurring mutations involving the same amino acids have been found in the $G_{i2}\alpha$ gene in adrenal cortical and ovarian neoplasms (50) (Table 2). This observation has not been confirmed in other studies (53), nor is the etiological significance of these $G_{i2}\alpha$ mutations clear. In contrast, activating $G_s\alpha$ mutations offer a plausible explanation for somatotroph and thyroid tumors because stimulation of the cAMP cascade by GHRH and TSH is known to promote cell proliferation and increased hormone secretion of the somatotroph and thyrocyte, respectively (54).

The McCune-Albright syndrome (MAS) is characterized by polyostotic fibrous dysplasia, cafe-au-lait skin hyperpigmentation, and autonomous endocrine hyperfunction (classically gonadotropin-independent precocious puberty, but acromegaly, hyperthyroidism, and adrenal cortical hyperfunction have also been described). The disorder is sporadic rather than inherited. Activating mutations of arginine 201 of the $G_s\alpha$ gene have been found in a mosaic distribution in tissues of subjects with MAS (55). This is consistent with the idea that a somatic mutation of $G_s\alpha$ occurs as an early postzygotic event. According to this hypothesis, a germ-line activating mutation of $G_s\alpha$ would be lethal, but an early somatic mutation, although nonlethal, would cause widespread disease manifestations reflecting the mosaic distribution of the

mutation. The increased cAMP formation caused by the mutation offers an explanation not only for the endocrine hyperfunction (see above), but also for the skin hyperpigmentation that is normally mediated by MSH acting through a $G_s\alpha$-coupled receptor to increase cAMP. Activating $G_s\alpha$ mutations have also been identified in affected bone from subjects with fibrous dysplasia (both polyostotic and monostotic), but the pathophysiology of this lesion requires further study (56). It appears that the time of occurrence of somatic $G_s\alpha$ mutations determines the nature and distribution of the manifestations. Mutations occurring very early in embryogenesis may cause pleiotropic, potentially lethal manifestations (57), whereas those occurring much later cause focal diseases, such as somatotroph tumors or monostotic fibrous dysplasia.

Speculations Concerning Future Developments

Discovery of other defects in G protein-coupled signal transduction in endocrine disorders

It is likely that additional endocrine (and nonendocrine) human diseases will be found to be caused by mutations in GPCR and G protein α-subunits. Many GPCR and several G proteins are involved in cell growth regulation. In transfection assays, overexpression of serotonin or muscarinic receptors causes cell transformation, as does expression of constitutively activated mutant forms of α_1-adrenergic receptors or of G_{i2}, G_q, G_{12}, and G_{13} α-subunits (58). To date, however, with the exception of $G_{i2}\alpha$, mentioned above, mutated forms of these genes have not been identified in naturally occurring human tumors. By analogy with the activated TSH receptor found in thyroid neoplasms, activating ACTH receptor mutations were sought, but not found, in adrenal cortical neoplasms (59). Likewise, in a series of nine pituitary thyrotroph tumors there was no evidence for activating mutations of the TRH receptor or of G_q, G_s, or G_{11} α-subunits (60). Some GPCR, such as somatostatin and D2-dopaminergic receptors, may act as negative growth regulators (*e.g.* in somatotrophs and lactotrophs, respectively). Somatic loss of function mutations of these GPCR genes or of the G proteins (G_i and G_o) to which they couple could lead to neoplasia, as would the loss of other tumor-suppressor genes. Such mutations of the D2-dopamine receptor were not identified in a series of prolactinomas (61); nonetheless, further studies of these and other endocrine tumors are warranted.

Further study of GPCR and G proteins is needed in nonneoplastic endocrine disorders as well. Although the clinical consequences of GPCR or G protein mutations are often quite predictable, this is not always the case. Loss of function mutations of the endothelin-B receptor, for example, cause Hirschprung disease through an as yet undisclosed mechanism (62). Transgenic and knock-out mouse approaches should be helpful in elucidating the pathophysiological consequences of such mutations. As discussed previously, some naturally occurring mouse mutants, such as *hyt* (16) and *little* (18), mimic human endocrine disorders (TSH and GHRH resistance, respectively). Naturally occurring MSH receptor-activating and -inactivating mutations cause hyper- and hypopigmentation, respectively, in mice (63). The ability to generate artificially mutant mice expands the range of disorders that can be studied in mouse models. Endothelin-B receptor knock-out mice show a phenotype strikingly similar to the human disorder (64). Knock-out of the β_3-adrenergic receptor in mice helps predict the potential consequences (or lack of consequences) of loss of function mutation of this receptor in humans (65). Knock-out of the mouse $G_{i2}\alpha$ gene unexpectedly caused an ulcerative colitis-like disease in null homozygotes (66). This and other surprising phenotypes associated with future knock-out models should prompt studies of signal transduction components in disorders not previously linked to abnormalities in G protein-coupled signal transduction.

Better studies are needed to help elucidate the role, if any, of more subtle abnormalities, including minor quantitative changes and differences in posttranslational modifications such as phosphorylation, in G protein-coupled signal transduction in human disease. The significance of naturally occurring polymorphisms in GPCR and G proteins in particular deserves further study. Certain TSH receptor polymorphisms (aspartate 36 to histidine and proline 52 to threonine) have been claimed to predispose to Graves' disease, but the importance of these differences has been questioned (42). Polymorphisms in the glucagon receptor (glycine 40 to serine) (67) and in the β_3-adrenergic receptor (tryptophan 64 to arginine) (68) have been identified and associated with type II diabetes and a reduced resting metabolic rate, respectively. In neither case, however, has the pathophysiological significance of the altered sequence been conclusively demonstrated.

Finally, it should be clear to the reader that defects in G protein-coupled signal transduction may involve more than defective GPCR or G protein α-subunits. This review has focused on these two components because they have been best studied and harbor most of the known mutations in endocrine disorders (indeed, for defects in G protein α-subunits in human disorders, it is principally $G_s\alpha$ that has been studied; Table 2). With the increasing appreciation of the importance of β/γ-subunits in signal transduction (4), they as well as G protein-regulated effectors and further downstream components will require analysis for possible disease-causing lesions. The pathophysiological importance of proteins, such as GPCR kinases, involved in agonist-dependent desensitization has already been demonstrated in transgenic mouse models (69), so that studies of these components should be particularly fruitful in disorders characterized by changes in sensitivity to GPCR agonists.

Implications for diagnosis and treatment

The identification of naturally occurring mutations of GPCR and G protein α-subunits as causes of endocrine diseases has already had major implications for understanding the structure and function of these signaling proteins. From an intellectual standpoint, defining critical receptor residues by identification of mutations causing an obvious phenotype such as NDI is much more efficient than random or saturation mutagenesis. The ability of certain mutations (*e.g.* residues in transmembrane helix 6 in the LH receptor; alanine 366 to serine in $G_s\alpha$) to cause constitutive activation provides

novel insights into the molecular basis for signal transduction (36, 49). The differing clinical consequences of certain mutations (*e.g.* the ability of activating mutations of $G_s\alpha$ to cause precocious puberty in both females and males, whereas activating LH receptor mutations trigger precocious puberty only in males) also provide valuable insights into normal physiological regulation. However, what are the implications of identifying GPCR and G protein mutations for diagnosis and treatment of endocrine disorders?

Unfortunately, as yet, the implications are rather limited, a situation similar to the general status of human genetic disorders despite the impressive recent progress in disease gene identification. For most endocrine disorders, functional diagnosis still depends on measurement of relevant hormone concentrations, and treatment still consists primarily of replacement of the deficient hormone(s) or correction by medical or surgical treatment of hormone hypersecretion. Genetic diagnosis does have immediate practical implications in certain conditions. In X-linked NDI, for example, it is now possible by V2-vasopressin receptor mutation analysis to determine whether a clinically unaffected female is a mutation carrier, and whether her male offspring are at risk for disease. At risk males can be tested immediately postnatally or, in theory, even prenatally to determine if they are affected, and if so, appropriate fluid replacement can be started immediately to avoid any dehydration (22). As most kindreds with X-linked NDI have distinct mutations, no simple diagnostic test is available, but the V2-vasopressin receptor gene is relatively small (~1.7 kilobases), and mutation analysis of it is straightforward in most molecular genetics labs. Mutation analysis of the CaR gene, which is much larger, is a more formidable task, but may be helpful in distinguishing FHH from primary hyperparathyroidism in hypercalcemic subjects (32). The distinction is not academic, because in FHH, parathyroidectomy is generally not indicated, whereas it generally is in hyperparathyroidism. As not all kindreds with FHH are linked to the CaR gene on chromosome 3q, and mutations have not always been identified even in kindreds linked to 3q, failure to find a CaR mutation does not exclude FHH (32).

Identification of constitutively activated $G_s\alpha$ in a subset of somatotroph tumors causing acromegaly and in involved tissues from subjects with MAS may be important in predicting responsiveness to treatment with somatostatin. *In vitro* studies of somatotroph tumors showed that those harboring $G_s\alpha$ mutations were much more likely to respond with a reduction in GH secretion than those lacking such mutations (70). Somatostatin has been effective *in vivo* in reducing GH hypersecretion in subjects with MAS (71). This suggests that although cAMP formation by adenylyl cyclase is being stimulated by constitutively activated $G_s\alpha$, it is still susceptible to inhibition by input from G_i proteins activated by somatostatin receptors. This also suggests that other agents capable of reducing cAMP formation could be effective in treating disorders caused by constitutive $G_s\alpha$- or G_s-coupled receptor activation. *In vivo* studies in transgenic mice have shown that inverse agonists can block constitutive β-adrenergic receptor activation (6). It is possible that inverse agonists could be developed specifically for other receptors whose constitutive activation causes disease. Eventually, with the development of safer and more efficient gene therapy methods, one can envision somatic gene replacement treatment for disorders caused by simple loss of function mutations and, possibly, treatment of disorders caused by activating mutations with appropriate dominant negative genes. These ideas may seem fanciful at present, but the idea that disorders such as MAS or FHH are due to point mutations in a G protein or GPCR might have seemed equally fanciful just a few years ago.

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